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In vitro Evaluation of Antibacterial Activities of Crude Extracts of *Withania somnifera* (Ashwagandha) to Bacterial Pathogens

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ABSTRACT

The present aim of study is to detect the antibacterial property in the extracts of *Withania somnifera*. The antibacterial activity of *Withania somnifera* was tested on clinically isolated bacterial pathogens, i.e., *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis* causing infections in human beings. Different solvents as ethanol, ethyl acetate, dichloromethane, hexane (from higher polarity to lower polarity) extracts was used for the study of antibacterial activity, by agar well diffusion method. The bacterial plates were prepared by nutrient agar. The extracts at different concentration from 10 to 40 mg mL^{-1} were loaded in the wells prepared in nutrient agar. Zone of inhibition was measured around the wells to check the antibacterial activity of extracts. Results showed the polar solvents to have higher antibacterial property in comparison to the nonpolar solvents. Relatively higher Minimum Inhibitory Concentration (MIC) were obtained for both gram positive bacteria *S. aureus, B. subtilis* and gram negative bacteria, *E. coli* and *P. aeruginosa*, with polar extract; however, less inhibitory effect was noted for nonpolar extracts. Ethyl acetate extract possesses great inhibitory activity for gram positive bacteria, *S. aureus* followed by *B. subtilis*. Among gram negative bacteria, highest inhibitory effect was observed with *P. aeruginosa* followed by *E. coli*. Antimicrobial activity of crude extract of *W. somnifera* were carried out to validate the use of traditional medicinal herbal and the results of this study tend to give credence to the common use of *W. somnifera* plant.

Key words: *Withania somnifera,* antibacterial activity, medicinal plants, minimum inhibitory concentration, agar well cup diffusion, herbal extracts, ashwagandha

INTRODUCTION

Multi-drug resistance is a world-wide problem, attributed to the extensive use of antibiotics, selection pressure on bacterial strains and lack of new drugs, vaccines and diagnostic aids. These shortcomings lead to an urgent global call for new antimicrobial drugs, particularly from natural resources. Majority of medicinal plant species are rich in biomolecule contents which can cope with health hazards and recently, antibacterial activity of many plant species have been reported Pandey and Mishra (2010). The genera *Withania somnifera* plays an important role in the indigenous medicine of South East Asia, e.g., in the Unani and Ayurvedic systems. The twenty-three known *Withania* species are widely distributed in the drier parts of tropical and subtropical zones, ranging from the Canary Islands, the Mediterranean region and northern Africa to Southwest Asia (Mirjalili *et al*., 2009). *Withania somnifera* has been used as an antioxidant,

adaptogen, aphrodisiac, liver tonic, anti-inflammatory agent and astringent and more recently as an antibacterial, antihyperplycemic and antitumoral, as well as to treat ulcers and senile dementia (Rastogi and Mehrotra, 1998). Most of its biological activities have been attributed to the presence of group of compounds referred as withanolides. The roots and leaves of *Withania* are used as drugs (Khanna *et al*., 2006). Various withanolides have been isolated from *W. somnifera*. Withaferin A and 3-β-hydroxy-2, 3 dihydro withanolide F show promising antibacterial, antitumour, immunomodulating and anti-inflammatory properties. It also possesses adaptogenic, cardiotropic, cardioprotective and anticoagulant properties (Rasool and Varalakshmi, 2006). *Withania somnifera* is widely claimed to have potent aphrodisiac, sedative, rejuvenative and life prolonging properties. The plant was traditionally used to promote youthful vigor, endurance, strength and health, nurturing the time elements of the body and increasing the production of vital fluids, muscle fat, blood, lymph, semen and cells. The similarity between these restorative properties and those of ginseng roots has led to ashwagandha roots being called Indian ginseng. It also helps counteract chronic fatigue, weakness, dehydration, bone weakness, loose teeth, thirst, impotency, premature ageing, emaciation, debility and muscle tension. The leaves of the plant are bitter in taste and used as an antihelmenthic (Mirjalili *et al*., 2009). Bruised leaves and fruits are locally applied to tumors and tubercular glands, carbuncles and ulcers. The roots are used as a nutrient and health restorative in pregnant women and old people. The roots are also used in constipation, senile debility, rheumatism, general debility, nervous exhaustion, loss of memory, loss of muscular energy and spermatorrhoea (Harikrishnan *et al*., 2008).

Withania somnifera contains active ingredients like steroidal alkaloids and lactones known as withanolides. Withaferin A and withanolide D are the two main withanolides that contribute to most of the biological activities of *Withania somnifera* (Mirjalili *et al*., 2009; Harikrishnan *et al*., 2008). In this study, we determine the antimicrobial activities of *W. somnifera* extract. Although a lot of work has been carried out on the medicinal applications of *W. somnifera,* there is still little information on the uses of the stem and leaves (Elsakka *et al*., 1990; Arora *et al*., 2004; Rajendran and Ramakrishnan, 2009). This study, therefore, provides information on the antibacterial activity (against the microorganisms causing skin, upper respiratory tract, gastrointestinal and urinogenital tract infection) of *W. somnifera* extract.

MATERIALS AND METHODS

The present study was done from March to May, 2010 in the Plant Biotechnology Laboratory, Centre for Biotechnology, University of Allahabad, Allahabad. Plantlets of *W. somnifera* was collected from the nursery in Allahabad.

Test organisms: Pure bacterial cultures (Table 1) were obtained from National Collection of Industrial Microorganisms (NCIM) National Chemical Laboratory, Pune and were maintained on nutrient agar (Rajendran and Ramakrishnan, 2009).

Extraction from *W. somnifera*: Whole plant of *W. somnifera* was washed thoroughly under running tap water, dried on paper towel, then kept in oven at 60°C for proper drying and finally crushed to fine powder in mixer grinder. The dried powder of the plant (10 g) was dissolved in 100 mL of ethyl acetate in soxhlet apparatus. The extract was collected after three days, filtered and kept in sterilized dark bottle. This procedure is repeated three times for proper extraction. The extracts were evaporated to dryness using rotary evaporator. A semisolid or dried crude extracts

Table 1: List of bacterial pathogens isolated from patients (1)

Bacteria	Diseases
Staphylococcus aureus	UTI, upper and lower respiratory tract infection, staphylococcal scalded skin syndrome (SSSS), septicarthritis, staphylococcal endocarditis (infection of the heart valves), pneumonia, skin infections (may occur as a commensal on human skin; it also occurs in nose frequently) such as pimple sand impetigo, meningitis, Toxic Shock Syndrome (TSS)
Bacillus subtilis	Human pathogen, it may contaminate food causes food poisoning *B. subtilis* produces the proteolytic enzyme subtilisin. *B. subtilis* spores can survive the extreme heating that is often used to cook food, and it is responsible for causing *ropiness*-a sticky, stringy consistency caused by bacterial production of long in spoiled bread dough
Escherichia coli	Urinary tract infection, cystitis and acute pyelonephritis
Pseudomonas aeruginosa	Urinary Tract Infection, upper and lower respiratory tract infection

of whole plant so obtained was resuspended in dimethyl sulphoxide (DMSO) to determine minimum inhibitory concentration (Ghosh *et al*., 2008). It was stored at 4°C for further studies. The residue was dried and the above procedure was followed for ethanol, dichloromethane and hexane also. The extractive values of ethyl acetate, ethanol, dichloromethane and hexane plant extracts were analysed for antimicrobial activity.

Determine minimum inhibitory concentration: MIC was determined by the broth dilution method (NCCLS, 2000). Different concentrations of whole plant in ethyl acetate, ethanol, dichloromethane and hexane (ranging from 10 mg mL^{-1} to 40 g mL^{-1}) were tested separately for each bacterium and inhibition zone of microbial growth in the plates containing tested solutions was judged by comparison with blank control plates. Minimum inhibitory concentration is defined as the lowest concentration of test samples that result in a complete inhibition of visible growth. Experiments were carried out in triplicate.

Antibacterial activities: The antibacterial susceptibility tests were carried out using agar diffusion method (Rajendran and Ramakrishnan, 2009; Mahesh and Satish, 2008; Perez *et al*., 1990; Kambizi and Afolayan 2008; Lokhande *et al*., 2007) which is routinely used in hospitals to test antimicrobial susceptibility for antibiotic-resistant bacteria, followed by the dilution method for products which possess a bioactivity. Plant extracts were delivered into well form lower to higher concentration and plates were incubated at 37°C for 24 h. The presence of zone of inhibition was regarded as the indicator of antimicrobial action and antimicrobial activity was expressed in terms of average diameter of the zone of inhibition measured in millimeter. Each test was carried out in triplicate.

RESULTS

Whole plant extracts of *W. somnifera* (ethyl acetate) tend to inhibit gram positive bacteria, *S. aureus* and *B. subtilis*. However, the inhibitory activity was very low in hexane extract (15 mm and no zone of inhibition for 10 mg mL^{-1}) in comparison to ethanol extract (no zone of inhibition-10 mm), dichloromethane extract (15 mm-no zone of inhibition). Same pattern was also observed with gram negative bacteria, *E. coli* and *P. aeruginosa*. Relatively higher MIC concentrations were obtained for gram negative bacteria *P. aeruginosa* with ethyl acetate extract.

Surprisingly, no inhibitory effect has been noted for hexane, this could be attributed to the extraction of active component of *W. somnifera* in ethyl acetate rather than hexane. Results show

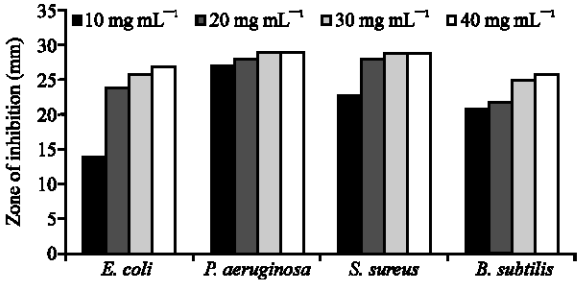

Fig. 1: Antibacterial activity of ethyl acetate extract of *Withania somnifera* at different concentration from 10 to 40 mg mL^{-1}

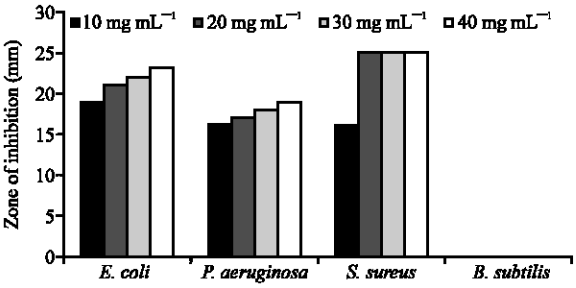

Fig. 2: Antibacterial activity of ethanol extract of *Withania somnifera* at different concentration from 10 to 40 mg mL^{-1}

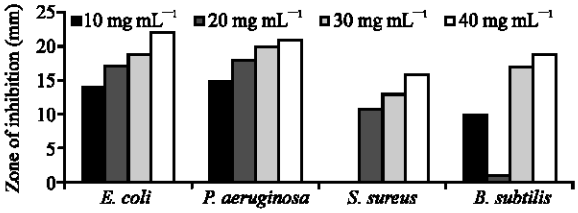

Fig. 3: Antibacterial activity of dichloromethane extract of *Withania somnifera* at different concentration from 10 to 40 mg mL^{-1}

that ethyl acetate extracts possess great inhibitory effect for gram positive bacteria, *S. aureus* followed by *B. subtilis* (Fig. 1). Among gram negative bacteria highest inhibitory effect was observed with *P. aeruginosa*, followed by *E. coli* (Fig. 1). Ethanolic extracts possess great inhibitory effect for gram positive bacteria, *S. aureus* whereas no zone of inhibition appeared for *B. subtilis* (Fig. 2). Among gram negative bacteria highest inhibitory effect was observed with *P. aeruginosa*, followed by *E. coli* (Fig. 2). In case of dichloromethane great inhibitory effect for gram positive bacteria *S. aureus* followed by *B. subtilis* and among gram negative bacteria highest inhibitory effect was observed with *P. aeruginosa*, followed by *E. coli* (Fig. 3) and in case of hexane extract zone of inhibition only appeared in *S. aureus* whereas no zone of inhibition appeared in case of *E. coli*, *P. aeruginosa* and *B. subtilis* (Fig. 4).

In this manuscript, we have reported that ethyl acetate extract of *Withania somnifera* plant has high antibacterial activity for gram negative as well as gram positive bacteria with a very low MIC.

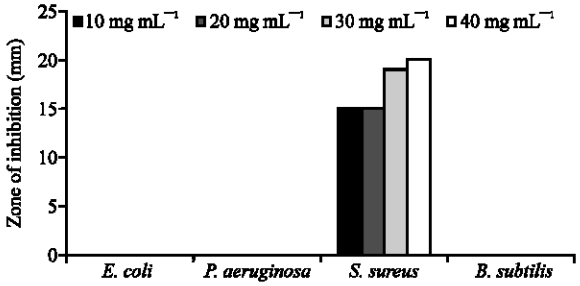

Fig. 4: Antibacterial activity of hexane extract of *Withania somnifera* at different concentration from 10 to 40 mg mL^{-1}

DISCUSSION

The present study strongly demonstrated that *W. somnifera* plant has potent antibacterial property. The above results indicate that both alcoholic as well as ethyl acetate extracts possessed strong antibacterial activity while hexane and dichloromethane fraction was not as effective against bacteria which shows the compounds were extracted in the polar solvents rather than nonpolar solvents (Owais *et al*., 2005). The methanolic extract was also inhibiting the growth of bacteria *P. aeruginosa, E. coli* and *S. aureus* than aqueous extract (Rajendran and Ramakrishnan, 2009). According to Mirjalili *et al*. (2009) the important compounds withaferin and withanolides were isolated from the methanolic extraction of the root *W. somnifera*. According to Arora *et al*. (2004), the methanolic extract of both leaves and root shows antibacterial activity, whereas, only root extract in hexane shows antibacterial activity. The previous findings also shows that the aqueous extract of *Withania* inhibit the growth of gram negative bacteria *N. gonorrhoea,* which also supports the result because water is the most polar solvent and the withanolides are extracted in the water properly (Kambizi and Afolayan, 2008). The methanolic extract of the *W. somnifera* also inhibit the growth of *B. subtilis, E. coli, P. fluorescens* and *S. aureus* (Mahesh and Satish, 2008). According to Choudhary *et al*. (1995), they used ethanol to extract withanolides and fraction it and did it spectroscopic studies to isolate the steroidal lactones that is withanolides. This finding also shows that the withanolides, steroidal lactones, are extracted in ethanol, methanol and ethyl acetate range of polar solvents which are potent inhibitor of bacterial growth.

CONCLUSION

The results of the viability assay have proved *W. somnifera* to hold excellent potential as an antibacterial agent. *W. somnifera* has withanolides which are steroidal lactones in nature and withaferin which makes a mucilaginous layer around the urinogenital, gastrointestinal and respiratory tract when consumed orally. The layers trap the microbial flora and make them unable to invade the system. Therefore, the bacteria cannot grow in the media containing *W. somnifera* extract. Thus, from the above investigation it can be concluded that the plant *W. somnifera* is a potential candidate for antimicrobial agent to treat diseases. Thus, further work can be carried out to isolate the exact active moiety responsible for the biological activity, characterize it and commercialize it.

REFERENCES

Arora, S., S. Dhillon, G. Rani and A. Nagpal, 2004. The *in vitro* antibacterial synergistic activities of *Withania somnifera* extracts. Fitoterapia, 75: 385-388.

Choudhary, M.I., Dur-E-Shahwar, Z. Parveen, A.Jabbar, I. Ali and Atta-ur-Rahman, 1995. Antifungal steroidal lactones from *withania coagulance*. Phytochemistry, 40: 1243-1246.

Elsakka, M., E. Grigorescu, U. Stanescu, U. Stanescu and V. Dorneanu, 1990. New data referring to chemistry of *Withania somnifera* species. Rev. Med. Chir Soc. Med. Nat. Iasi, 94: 385-387.

Ghosh, A., B.K. Das, A. Roy, B. Mandal and G. Chandra, 2008. Antibacterial activity of some medicinal plant extracts. J. Nat. Med., 62: 259-262.

Harikrishnan, B., P. Subramanian and S. Subash, 2008. Effect of *Withania somnifera* root powder on the levels of circulatory lipid peroxidation and liver marker enzymes in chronic hyperammonemia. E-J. Chem., 5: 872-877.

Kambizi, L. and A.J. Afolayan, 2008. Extracts from *Aloe ferox* and *Withania somnifera* inhibit *Candida albicans* and *Neisseria gonorrhoea*. Afr. J. Biotechnol., 7: 12-15.

Khanna, P.K., A. Kumar, A. Ahuja and M.K. Kaul, 2006. Biochemical composition of roots of *Withania somnifera* (L.) dunal. Asian J. Plant Sci., 5: 1061-1063.

Lokhande, P.D., K.R. Gawai, K.M. Kodam, B.S. Kuchekar, A.R. Chabukswar and S.C. Jagdale, 2007. Antibacterial activity of extracts of *Piper longum*. J. Pharmacol. Toxicol., 2: 574-579.

Mahesh, B. and S. Satish, 2008. Antimicrobial activity of some important medicinal plant against plant and human pathogens. World J. Agric. Sci., 4: 839-843.

Mirjalili, M.H., E. Moyano, M. Bonfill, R.M. Cusido and J. Palazon, 2009. Steroidal Lactones from *Withania somnifera*, an ancient plant for novel Medicine. Molecules, 14: 2373-2393.

NCCLS, 2000. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. 5th Edn., National Committee for Clinical Laboratory Standards, Approved Standard M7-A5, Wayne, PA, USA.

Owais, M., K.S. Sharad, A. Shehbaz and M. Saleemuddin, 2005. Antibacterial efficacy of Withania somnifera (ashwagandha) an indigenous medicinal plant against experimental murine salmonellosis. Phytomedicine, 12: 229-235.

Pandey, R. and A. Mishra, 2010. Antibacterial activities of crude extract of *Aloe barbadensis* to clinically isolated bacterial pathogens. Applied Biochem. Biotechnol., 160: 1356-1361.

Perez, C., A. Pauli and P. Bazerque, 1990. An antibiotic assay by agar-well diffusion method. Acta Biol. Med. Exp., 15: 113-115.

Rajendran, N.K. and J. Ramakrishnan, 2009. *In vitro* evaluation of antimicrobial activity of crude extracts of medicinal plants against multi drug resistant pathogens. Biyoloji Bilimleri Araþtýrma Dergisi, 2: 97-101.

Rasool, M. and P. Varalakshmi, 2006. Immunomodulatory role of *Withania somnifera* root powder on Experimental induced Inflammation: An *in vivo* and *in vitro* study. Vascular Pharmacol., 44: 406-410.

Rastogi, R.P. and B.N. Mehrotra, 1998. Withania Somnifera: Alternative Medicine Review. Vol. 6, Central Drug Research Institute, Luchnow and National Institute of Science Communication, New Delhi.